

(d) processing the progeny seed of step (c) to obtain seed oil containing petroselinic acid.

**REMARKS**

The specification has been amended to reflect the fact that Figure 3 was inadvertently omitted from the original filing of the application. References to the missing figure have been deleted. The results of the data shown in the Figure 3 are stated in the original specification. No new matter has been added.

Claims 27-45 are currently pending.

Claim 34 has been rewritten to be an independent form to address the objection raised on page 2 of the Office Action. No new matter has been added.

The claims were rejected under 35 U.S.C. 101 as lacking a specific (or credible, or well established) asserted utility. It is alleged on page 3 of the Office Action that "the specification does not provide any evidence to support the claim that this sequence would have delta-4-16:0-ACP desaturase activity. While the specification discloses methods for transforming plants with sequences and analyzing for seed oil fatty acids, there is no indication that any of these plants were transformed with the sequence that is claimed." (page 3, lines 3-7 of the Office Action.) Attention is kindly invited to Example 8 of the specification on pages 31-33. Example 8 describes the introduction of the English ivy delta-4-16:0-ACP desaturase (ehh1c.pk002.f22, SEQ ID NO:1) into a plant expression vector, and the subsequent transformation and expression of this construct in tobacco cells. The results are stated as follows:

--Expression of the cDNA for EST ehh1c.pk002.f22 in the resulting kanamycin resistant tobacco callus was confirmed by PCR analysis using the oligonucleotides described above and template consisting of first-strand cDNA prepared from RNA extracted from the transgenic callus. To determine the *in vivo* activity of the diverged acyl-ACP desaturase encoded by the cDNA for EST ehh1c.pk002.f22, the fatty acids composition of the transgenic tobacco callus was examined by gas chromatography. Fatty acid methyl esters were prepared by homogenization of the transgenic tobacco callus in 1% (w/v) sodium methoxide in methanol using methods described by Hitz et al. (1994) *Plant Physiol.* 105:635-641. The recovered fatty acid methyl esters were then analyzed using a Hewlett-Packard 6890 chromatograph fitted with an Omegawax 320 column (30 m x 0.32 mm inner diameter; Supelco). The oven temperature was programmed from 220°C (2 min hold) to 240°C at a rate of 20°C/min. The transgenic tobacco callus expressing the diverged *Hedera helix* acyl-ACP desaturase was found to contain a hexadecenoic acid (16:1) isomer and a

octadecenoic acid (18:1) isomer that were absent from untransformed callus. These two isomers each accounted for 2 to 5% (w/w) of the total fatty acids of the transgenic tobacco callus. Gas chromatography-mass spectrometry (GC-MS) was performed to determine the double bond positions of the novel 16:1 and 18:1 isomers. For these analyses, fatty acid methyl esters were converted to dimethyl disulfide derivatives using the method described by Yamamoto, K., Shibahara, A., Nakayama, T., Kajimoto, G. (1991) *Chem. Phys. Lipids* 60:39-50. Dimethyl disulfide derivatives were analyzed by GC-MS using a Hewlett Packard 6890 gas chromatograph interfaced with a Hewlett Packard 5973 mass selective detector. Samples were resolved with a HP-INNOWax column (Hewlett Packard) (30 m x 0.25 mm inner diameter), and the oven temperature was programmed from 185°C (5 min hold) to 237°C at a rate of 7.5°C/min. The resulting mass spectrum of the dimethyl disulfide derivative of the novel 16:1 methyl ester contained diagnostic ions consistent with that of a  $\Delta^4$  isomer (Fig. 3A). In addition, the mass spectrum of the dimethyl disulfide derivative of the novel 18:1 methyl ester in the transgenic tobacco callus contained diagnostic ions consistent with that of petroselinic acid, the 18:1 $\Delta^6$  isomer (Fig. 3B). These results thus indicate that the diverged acyl-ACP desaturase corresponding to the cDNA for EST ehh1c.pk002.f22 is associated with petroselinic acid synthesis. Based on the biosynthetic pathway for petroselinic acid previously described in Umbelliferae species [Cahoon, E.B. and Ohlrogge, J.B. (1994) *Plant Physiol.* 104:827-844], the *Hedera helix* diverged acyl-ACP desaturase is likely a  $\Delta^4$ -specific palmitoyl (16:0)-ACP desaturase. This is consistent with the presence of the novel 16:1 $\Delta^4$  isomer in the transgenic tobacco callus (Fig. 3A). The 16:1 $\Delta^4$  isomer bound to ACP likely serves as the biosynthetic precursor for petroselinic acid (18:1 $\Delta^6$ ), as described in Umbelliferae species [Cahoon, E.B. and Ohlrogge, J.B. (1994) *Plant Physiol.* 104:827-844]. --

Figure 3 referred to in the specification was inadvertently omitted from the original application. A copy of the figure is submitted as Appendix A herein to provide additional support for the statements in the specification.

In view of foregoing discussion and results, it is respectfully submitted that SEQ ID NO:1, and its corresponding translation product SEQ ID NO:2, does encode an English ivy delta-4-16:0-ACP desaturase. Accordingly, withdrawal of the rejection of the claims under 35 U.S.C. 101 is respectfully requested.

Claims 27-45 were rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

It is believed that the discussion above with respect to the rejection of the claims under 35 USC §101 is equally apposite here. Accordingly, withdrawal of the this ground of rejection is respectfully requested.

Claims 27, 34, and 36-45 were rejected under 35 U.S.C. 102(b) as being anticipated by Cahoon et al (PNAS 89: 11184-11188, 1992). The alignment which accompanied the Office Action was generated using the GenCore alignment method showing a 75% identity between applicant's SEQ ID NO:2 and the coriander sequence (A47245) previously disclosed by Cahoon et al.

The alignment shown in Appendix B, attached hereto, was performed with Clustal under the default parameters, as outlined in the specification (page 25, lines 11-17.) SEQ ID NOs: 2 and 6 are shown aligned versus the gi 417819 coriander sequence in the specification (SEQ ID NO:7) and the A47245 sequence used in the examiner's alignment. The two art sequences are identical. The percent identity between SEQ ID NO:2 and the art sequences, using the Clustal method, is 73.8%, as shown in the Pair Distance table in Appendix B, and in Table 4 of the specification. The Clustal method of alignment is recited in the claims of the instant invention. The default parameters used in the alignment are found in several places within the specification, including page 25, lines 13-17. Accordingly, it is respectfully submitted that the claimed invention of 75% identity by the Clustal alignment method does not read on the art. In light of this evidence it is respectfully requested that the rejection under 35 U.S.C. 102(b) be withdrawn.

It is respectfully submitted that the claims are now in form for allowance, which allowance is respectfully requested.

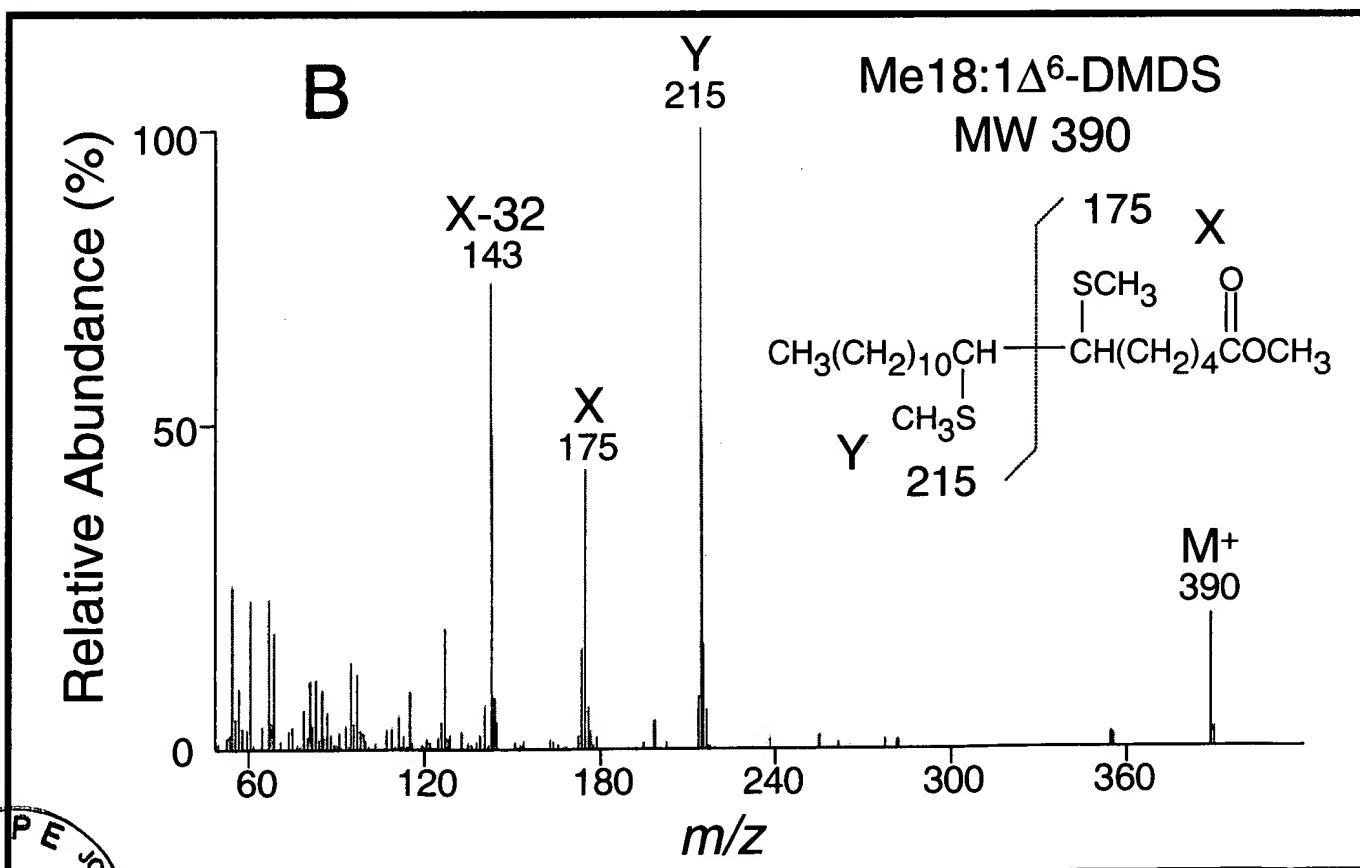
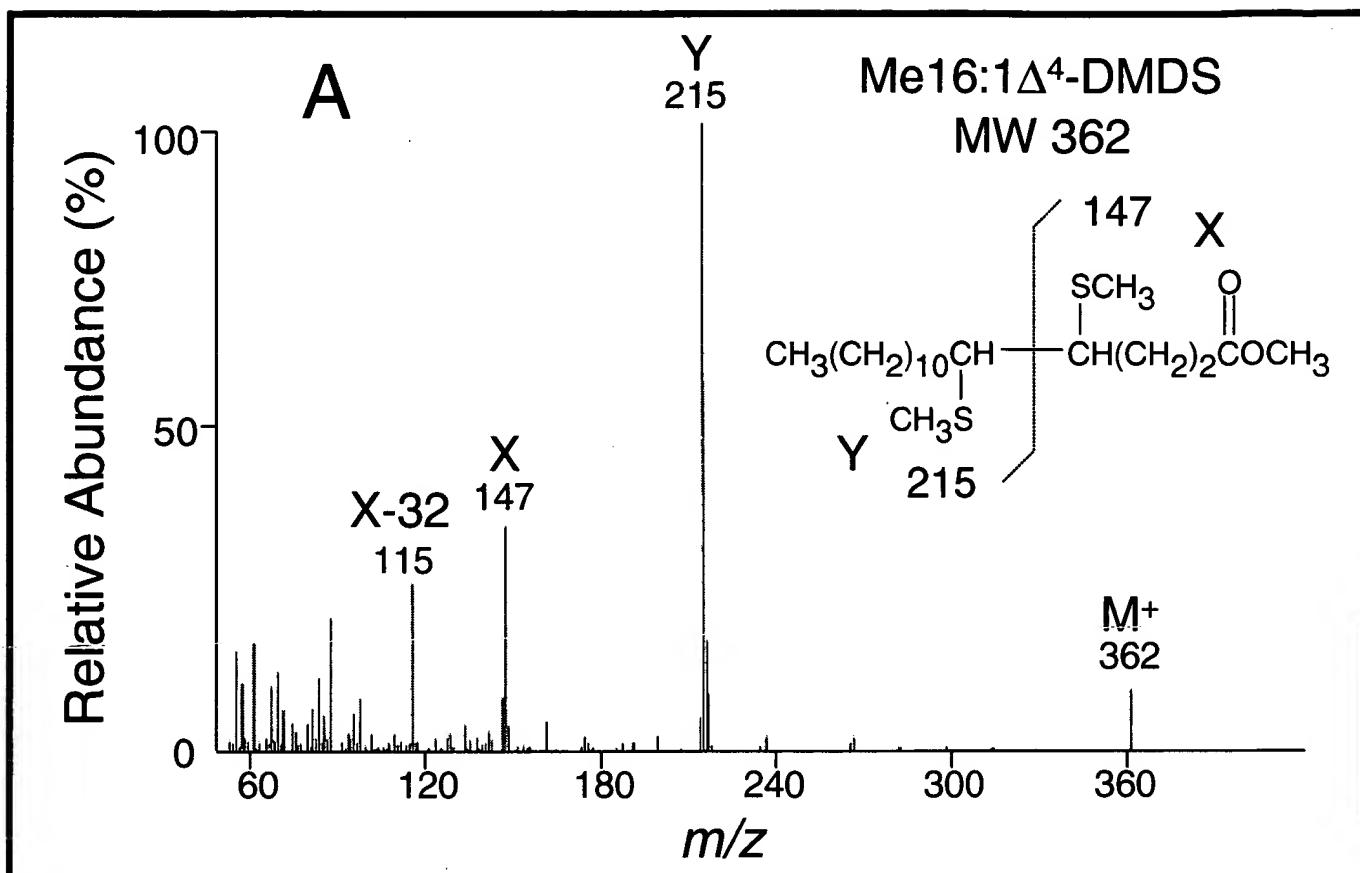
A Petition for a two (2) month extension of time accompanies this response along with Appendices A and B as discussed above.

Please charge any fees or credit any overpayment of fees which are required in connection with the filing of this Response and Petition for Extension of Time to Deposit Account No. 04-1928).

Respectfully submitted,

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Dated: June 30, 2003



## APPENDIX B

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#NEXUS
[!Alignment of:]
[Sequence Start End Sequencetype]
[SEQ ID NO 6 (1 > 137) PROTEIN]
[SEQ ID NO 2 (1 > 394) PROTEIN]
[417819 (1 > 385) PROTEIN]
[A47245 (1 > 385) PROTEIN]

begin data;
dimensions ntax=4 nchar=398;
format datatype=Protein interleave gap=- missing='.';
matrix

SEQ ID NO 6 MA-----SVTASSISFTSI--ASSLKQ
SEQ ID NO 2 MALKLN----FQCKKNHPAAFAKSPLPVTRVSSPRVFMASTVNSNSMVLNDNLKSPPNLQV
417819 MAMKLNALMTLQCPKRN--MFTRIAPPQAGRVRSKVSMASTLHASPLVFDKLKAGR---
A47245 MAMKLNALMTLQCPKRN--MFTRIAPPQAGRVRSKVSMASTLHASPLVFDKLKAGR---

SEQ ID NO 6 NQGLAKSSISL--SVNG-----KSFRS---
SEQ ID NO 2 THSMPPQKLEIFKSLDDWARNNVLILHLSVEKSWQPQDYLPPVSDGEEQVRELRERAK
417819 -----PEVDELFNSLEGWARDNILVHLKSVENSWQPQDYLPPTSDAFEDQVKEMRERAK
A47245 -----PEVDELFNSLEGWARDNILVHLKSVENSWQPQDYLPPTSDAFEDQVKEMRERAK

SEQ ID NO 6 -----
SEQ ID NO 2 EIPDDYFVVLVGDMITEEALPTYMSMLNRCDGKIDETGAEPSAWAMWTRAWTAEEENRHD
417819 DIPDEYFVVLVGDMITEEALPTYMSMLNRCDGKIDDTGAQPTSWATWTRAWTAEEENRHD
A47245 DIPDEYFVVLVGDMITEEALPTYMSMLNRCDGKIDDTGAQPTSWATWTRAWTAEEENRHD

SEQ ID NO 6 -----LRLLSAPLRFRVSCAAKPAT-----
SEQ ID NO 2 LLNKYLQLSGRVDMRMIEKTIQYLGFIYTSFQERATFISHANTAK
417819 LLNKYLQLSGRVDMRMIEKTIQYLGFIYTSFQERATFISHANTAK
A47245 LLNKYLQLSGRVDMRMIEKTIQYLGFIYTSFQERATFISHANTAK

SEQ ID NO 6 -----VDKVC-----EIVRKQLALPAD
SEQ ID NO 2 LAQHYGDKNLAHICGSIASDEKRHATAYTKIVEKLAEPDTTIAFADMMRKKITMPAH
417819 LAQHYGDKNLAQVCVNIASDEKRHATAYTKIVEKLAEPDTTIAFSDMMRKKIQMPAH
A47245 LAQHYGDKNLAQVCVNIASDEKRHATAYTKIVEKLAEPDTTIAFSDMMRKKIQMPAH

SEQ ID NO 6 SAVTGESK----FAALGAD----SLDTVEIVMGLEEEF----GISVEEESAQT-IA
SEQ ID NO 2 LMYDGSDDELLFKHFTAVAQRVXVYSAODYCDILEFLVDKWNVERLTGLSDEGRKAQEYVC
417819 AMYDGSSDDMLFKHFTAVAQQIGVYSAWDYCDIIDFLVDKWNVAKMTGLSGEGRKAQEYVC
A47245 AMYDGSSDDMLFKHFTAVAQQIGVYSAWDYCDIIDFLVDKWNVAKMTGLSGEGRKAQEYVC

SEQ ID NO 6 TVQDAADLIEKLVEKKE-----
SEQ ID NO 2 ELGPKIRRVEEKVQGKEKKKAEPVFSWIFNRELKI
417819 SLAAKIRRVEEKVQGKEKK--AVLPVAFSWIFNRQIII
A47245 SLAAKIRRVEEKVQGKEKK--AVLPVAFSWIFNRQIII

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### Pair Distances of Untitled ClustalV (PAM250)

Percent Similarity in upper triangle

Percent Divergence in lower triangle

	SEQ ID NO 6	SEQ ID NO 2	417819	A47245	
SEQ ID NO 6	***	14.6	14.6	14.6	SEQ ID NO 6
SEQ ID NO 2	232.0	***	73.8	73.8	SEQ ID NO 2
417819	191.3	26.6	***	100.0	417819

